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Crop Genetic Responses to Management: Evidence of Root–Shoot Communication

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CONTENTS

A number of successful sustainable agriculture systems for vegetable production have been developed in recent years for the mid-Atlantic states of the USA. Of particular interest is the use of an annual legume, hairy vetch, which has proved to be a beneficial cover crop because it fits well into different cropping rotations, is capable of high nitrogen fixation, and produces substantial biomass (Kelly et al., 1995; Teasdale and Abdul-Baki, 1997; Araki and Ito, 2004). In the UK, an assessment of agricultural practices recently concluded that no-till agriculture is potentially more beneficial than others (Trewavas, 2004). Thanks to methods for molecular biological investigation, we are beginning to understand how and why such practices are contributing to better crop performance and more sustainable agricultural production, by affecting the expression of favorable genetic potentials in crop plants.

This chapter reviews some recent research that indicates some previously unrecognized ways in which the management of plants, soil, and nutrients affects crop growth. These connections have been apparent in gross observational terms and have been reported in measured correlations, but without a good understanding of the mechanisms involved. More research is needed to establish the extent and malleability of the mechanisms now being mapped out. However, work so far illuminates part of the complex web of plant–soil interactions that determine agricultural success. Although microbial partners in these interactions have not been studied yet, they are likely to have some roles in this dynamic process as well. Here we report what is presently known, expecting that further research in this area will expand this knowledge.

15.1 Hairy Vetch-Vegetable Associations

When planted in mid-September and grown through the winter, hairy vetch is ready to be terminated before summer vegetables such as tomatoes and peppers are transplanted in the field in late spring (Teasdale and Abdul-Baki, 1997). We note that for fall vegetables such as broccoli, a mixture of forage soybean (*Glycine max* L.) and foxtail millet (*Setaria italica* L. P. Beaver) that can be grown over the summer has proved to have similar effects (Abdul-Baki et al., 1997).

Yields of tomatoes that are grown in hairy vetch mulch over several years have averaged 20–40% higher than those produced by conventional methods, which usually include the use of black polyethylene mulch (Teasdale and Abdul-Baki, 1997; Whitehead and Singh, 2003). Further, when mulching with hairy vetch, soil fertility has substantially improved over time, even with lower input of chemical fertilizer. This finding has presented a challenge to agricultural scientists to explain why hairy vetch mulch accompanied by less inorganic fertilization gives better results than the "modern" system that employs polyethylene mulch and high levels of synthetic fertilizer application. If this alternative system warrants confidence, there are significant environmental benefits to be gained from greater reliance on organic inputs to expand horticultural production.

Various benefits seen from using cover-crop mulches in cropping systems can directly enhance crop productivity and quality — the retention of longevity and greenness in the leaves (Teasdale and Abdul-Baki, 1997; Kumar et al., 2004), less pest infestation, and enhanced tolerance or resistance to disease (Mills et al., 2002). Economic studies have supported the sustainability and superiority of this alternative system over the conventional system (Kelly et al., 1995; Lu et al., 2000). However, despite these evident advantages of alternative farming practices, their spread has been slow. This may be partly because it is thought that the scientific basis for alternative agriculture is weak (Trewavas, 2001). Until more is known about the mechanism(s) that underlie these beneficial aspects of legume cover crops, many producers may be hesitant to adopt such alternative farming practices. The desirable performance of cover-crop, mulch-grown vegetables has presented an opportunity to explore how the environment can control gene function. Recent research (Kumar et al., 2004, 2005) has shown that in tomatoes grown after a hairy-vetch cover crop, a highly organized and specific network of genes is modulated in the crop, making it possible for the same or reduced inputs to produce greater outputs, something that agriculturalists generally desire.

15.2 Activation of Specific Genes in Crop Associations

Long duration of foliage greenness in plants is an indication of delayed senescence (aging) of their leaves. In all plants' development, a process of leaf senescence will at some point invariably follow that of leaf expansion; however, the timing can vary. Senescence is generally thought to result from the rapid degradation of certain essential proteins in the plant, which accumulate during leaf development and expansion. These include, particularly, rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase), a protein essential for photosynthesis and N remobilization; glutamine synthetase (GS), which plays a major role in N conversion and C utilization for the synthesis of amino acids, the building blocks for all proteins; the enzyme ATPase, and chlorophyll-binding proteins in the chloroplast, cytb559, and plastocyanin, all important for photosynthesis and converting harvested chemical energy into food (Roberts et al., 1987; Mehta et al., 1992). Further description of their functions is provided below.

The onset of degradation of rubisco and other proteins during leaf senescence is thought to provide nitrogen in the form of amino acids that are released to support the growth of other plant tissues, for example, young or developing leaves and fruits (Thimann, 1980). The stable accumulation and retention of these compounds in a developed leaf is thus associated with leaf longevity. Of special interest is the persistence in leaf tissues of rubisco and GS, which are critical for photosynthesis and the carbon–nitrogen (C/N) metabolism of any crop (Makino et al., 1984).

It is therefore significant that in the leaves of tomato plants grown in hairy-vetch mulch, we find that the transcripts as well as protein levels of rubisco and cytosolic GS remain higher and stable for a longer duration than in plants grown in the conventional black-polyethylene mulch. On the other hand, the levels of gene transcripts for a nitrogen-responsive transporter, ATPase, chlorophyll-binding proteins, and many other chloroplast-localized proteins were not found to be much different (Kumar et al., 2004). Thus, the hairy-vetch-based production system specifically induces the expression of genes that are associated with prolonged plant growth.

Similarly, it has been found that genes contributing to plants' defense against pests and diseases are activated in tomatoes grown with vetch mulch. Basic chitinase (Broglie et al., 1986) and osmotin (Liu et al., 1994) are among the defense proteins that enable plants to resist attacks by pests including pathogenic fungi. The gene transcripts for these two proteins are highly expressed — and their proteins accumulate for a more prolonged period — in tomatoes that were grown in hairy-vetch mulch compared to those grown in black polyethylene. It is interesting that tomatoes grown in either hairy vetch or with black polyethylene were found (Kumar et al., 2004) not to be different in the plant disease-resistance pathways signaled via nitric oxide, a previously documented mechanism for defense against pathogen infection (Durner and Klessig, 1999).

Chaperones are a class of proteins that stabilize native proteins, prevent the aggregation of denatured proteins, and catalyze proper folding of proteins (Pelham, 1986; Hammond and Helenius, 1995). Two such proteins — heat shock protein-70 (hsp70) (Li et al., 1999), and ER binding protein (BIP) (Kalinski et al., 1995) — which are normally activated during periods of plant stress were found to be present at higher steady-state levels (both for transcripts and for proteins) in tomatoes grown in the hairy-vetch mulch compared to those grown in black-polyethylene mulch. The higher stability of hsp-70 and BiP in vetch-mulched tomato plants suggests that these proteins continued to be recruited and maintained in plant tissues longer, which helps prolong the longevity of these plants compared to those grown with black polyethylene (Kumar et al., 2004).

One of the promoters of aging and senescence in plants is the hormone ethylene, which also regulates defensive processes in some plants (Boller, 1991; Lund et al., 1998; Mattoo and Handa, 2004). A key enzyme that enables a plant to synthesize ethylene is known as 1-aminocyclopropane-1-carboxylate (ACC) synthase. There are several forms of this enzyme (Rottmann et al., 1991; Fluhr and Mattoo, 1996). We have found that the gene transcripts for one of them, ACS6 — and for a senescence-associated gene, SAG12 (Noh and Amasino, 1999) — accumulate slowly in hairy-vetch-grown tomatoes compared to the black-polyethylene-grown plants (Kumar et al., 2004). This contrasts with the opposite pattern of accumulation for a receptor protein kinase (CRK) that senses (Papon et al., 2002) the level of cytokinin, an anti-senescence and pro-growth hormone. That CRK is higher in the hairy-vetch-grown tomato suggests that hormonal "cross-talk" and more signaling are key factors associated with the beneficial attributes we find characteristic of plants grown on hairy-vetch mulch.

The activation and retention of gene transcripts for the following proteins define the signature of tomato plants cultivated in the hairy-vetch-based alternative agriculture (Kumar et al., 2004, 2005). We note what each contributes to plant metabolism and growth to indicate the intricacy and complexity of the various processes that plant and soil management practices are affecting:

- N-responsive GS1, important for C/N metabolism and signaling;
- rubisco, one of the most abundant proteins in the biosphere, critical for C fixation;
- nitrite reductase, an enzyme that converts nitrite to ammonia, facilitating N utilization and reducing nitrite toxicity;
- glucose-6-phosphate dehydrogenase, an enzyme that contributes to N-use efficiency;
- two chaperone proteins, heat shock protein-70 and ER lumenal binding protein, that are important for keeping other proteins in an active state;
- anti-fungal proteins, chitinase and osmotin, that provide a defense against pests and are binding partners for cytokinin, a growth-regulating hormone;
- cytokinin-responsive receptor kinase, a protein that determines cytokinin import into the leaf tissue;
- GA_{20} oxidase, an enzyme involved in signaling via another class of plant-growth hormones known as gibberellins (GA), discussed in the preceding chapter.

These studies have shown the existence of a specific interface between hairy-vetch mulch and the tomato plant that results in a fundamentally distinct expression-profile of gene transcripts and proteins in the tomato leaf which affect the growth and longevity of plants being raised on this leguminous mulch.

15.3 More Than Just Nitrogen Is Involved

An easy assumption could be that hairy-vetch residue has its beneficial effect on the growth of tomato plants, and even on the differential gene expression presented in the previous section, through an increased provision of nitrogen, since hairy vetch as a legume has known capacity to fix N (Araki and Ito, 2004). However, this is not an adequate explanation. First, the hairy-vetch-grown tomato plants received less total N input, 100 kg ha^{-1} (in the form of urea) compared to 200 kg ha^{-1} supplied to those plants grown in black polyethylene beds (Kumar et al., 2004; see also Abdul-Baki et al., 1997). Second, there was no significant correlation between total leaf N content and the differences observed in disease onset (or severity) and the senescence index as a function of the mulch used.

Although it is clear that differential gene expression described in the previous section is reminiscent of C/N signaling, because of the types of genes involved and the nature of their expression, hairy-vetch-based effects on tomato leaves represent a different biochemical mode of regulation. For example, short-term exposure of plants to applied N normally results in the activation of senescence-associated protein (SAG12) and the nitrate transporter (Wang et al., 2000); but this did not occur with hairy-vetch-grown tomato (Kumar et al., 2004). Similarly, exposure to a high level of N causes a reduction in osmotin transcripts in Arabidopsis (Wang et al., 2000), whereas in the hairy-vetch production system their transcripts actually accumulate.

The question arises: what are the other factors that work in conjunction with C/N signaling to produce the demonstrably beneficial effects in crops grown on a cover-crop mulch? Another contributing factor could be the type of soil microbes that are enriched in the cover-crop/tomato root association. It is interesting to note that some of the agronomic and physiological effects we have seen with hairy-vetch/tomato production system have been documented in Egypt with rice that is grown in association with berseem clover, also a legume (Chapter 8). The observations of Dazzo and Yanni, based on N-balance data, suggested that the benefit of rotating rice with clover was not due to an increased availability of soil N, from mineralization of the biologically-fixed, N-rich clover crop residues. As in our studies with tomato grown with a lower N fertilizer input, they obtained higher rice grain yields with less dependence on chemical fertilizer inputs, depending instead on certain strains of endophytic rhizobia as inoculants for the rice crop.

Of further significance for our discussion here, Dazzo and Yanni found that rice plants developed expanded root architectures when inoculated with rhizobial endophytes, which could make them better "miners" of other nutrients. They report higher concentrations of N, P, K^+, Na^+, Ca^{+2}, Mg^{+2}, and Zn^{+2} in rice inoculated with the endophytes as compared to plants that remained uninoculated (see Figure 8.6 and Section 15.6 below).

We have found that cover crops differ considerably in their ability to "mine" soil nutrients. For instance, a comparison of hairy-vetch residue vs. rye residue in terms of their respective abilities to accumulate minerals showed the former to be richer in P, B, Mn^{+2}, Mg^{+2}, Ca^{+2}, Fe^{+2}, and Zn^{+2} as well as in N (Abdul-Baki and Mattoo, unpublished data). At the same time, no significant differences were found in K, S, and Cu. What effect, if any, these differences in mineral nutrient availability between the two cover crops have on the ability of tomato plants to take these up and accumulate them in the foliage and fruit has not been assessed.

Dazzo and Yanni suggest an interesting scenario based on these findings where the nutritional value of grain could be enhanced, for example, for an essential micronutrient such as zinc. Whether specific microorganisms associated with the rhizosphere of the cover crop production system are responsible for the robust root architecture of tomato plants and/or assist in the acquisition of selected mineral nutrients as in the case of rice–endophyte association is an important subject that remains to be studied.

15.4 Root-to-Leaf Signals via Hormones

Our findings suggest that root physiology and hormonal signaling contribute to the delayed senescence of plants cultivated under leguminous cover-crop mulch. Hairy-vetch-based cultivation leads to more robust and larger spread of roots (Sainju et al., 2000), which contributes to higher yields of the crop. Of relevance for the mechanisms involved in hairy-vetch-based cropping is the fact that root growth regulates the synthesis of cytokinin, a plant hormone whose decrease is associated with initiation of senescence in plant organs (Nooden et al., 1997; Smart et al., 1991; Sakakibara et al., 1998; Hwang and Sheen, 2001).

Conversely, a continued supply of cytokinin from the roots to the upper parts of a plant should delay senescence. This seems to be the case with the tomato plants cultivated under hairy-vetch mulch, indirectly indicated by higher measured levels of an indicator gene, the cytokinin receptor protein kinase, CRK (Papon et al., 2002; Kumar et al., 2004). Cytokinin is known to inhibit the accumulation of senescence-enhancing genes (Noh and Amasino, 1999).

Another facet of interactive C/N metabolism and cytokinin signaling may be a factor in the coordination of delayed senescence of plants cultivated under hairy-vetch mulch with their greater tolerance to disease (Mills et al., 2002; Kumar et al., 2004). Cytokinin signaling has been implicated in plant–microbe interactions involving rhizobacteria (Ryu et al., 2003), while it is known that the engineered accumulation of cytokinins induces several defense-related genes including basic chitinase (Memelink et al., 1987) and osmotin (Thomas et al., 1995). Higher and steady accumulation of transcripts and protein of these two antifungal defense proteins, chitinase and osmotin, in hairy-vetch-grown tomato (Kumar et al., 2004) indicates that cytokinin signaling not only regulates senescence but also disease tolerance. Chitinase is an enzyme that degrades chitin, a polysaccharide component of the gut linings of insects and in the cell walls of fungi. Chitinolytic activity makes these enzymes potent anti-insect and anti-fungal agents, and their occurrence may have implications in the organism-to-organism interactions in the ecosystem (www.glfc.forestry.ca/frontline/bulletins/bulletin_no.18_e.html).

Our studies thus raise some important larger questions. How do hairy-vetch-grown tomatoes coordinate enhanced cytokinin and C/N signaling to cause delayed leaf senescence and greater tolerance of pests? In these plants, what induces and maintains cytokinin synthesis in the roots, and what regulates the root-to-leaf and leaf-to-root communications? Research is only beginning to get some grasp on these issues.

Another possible factor for some of the observations made with hairy-vetch-grown tomato could be their difference from the black-polyethylene-grown plants in the pace of growth. In the field in spring, soils stay cooler under vetch mulch than under polyethylene, which results in slower growth of tomatoes initially, in regard to root length and aboveground. Eventually they develop a more robust leaf area around the beginning of fruit maturity. Rapid tomato growth in polyethylene mulch could lead to faster accumulation of cytokinins in leaf tissue, and this can signal a feedback mechanism to shut down further cytokinin production, particularly when the levels of the cytokinin-binding partners is low in black-polyethylene-grown tomato. In turn, this would bring the polyethylene-grown tomato into reproductive mode sooner and cause earlier senescence under black-polyethylene mulch.

15.5 Possible Mechanisms Involved

The following proposed outline could account for the observed metabolic and genetic regulation in hairy-vetch-based alternative agriculture. For reasons yet to be fully understood, hairy-vetch cultivation of tomatoes generates a healthier and more robust root system as well as a higher steady-state level of cytokinins. It is understandable that a steady flow of cytokinins from the root to the leaf would signal for enhanced and stable levels of basic chitinase and osmotin. Both these proteins provide a defense against insect pests by binding to actin, thereby causing cytoplasmic aggregation (Takemoto et al., 1997). It is also known that basic chitinase and osmotin are binding partners for cytokinins (Kobayashi et al., 2000).

As a result of the tripartite combination of cytokinins, basic chitinase and osmotin, the latter two defense proteins would remain stable for a longer duration, which would maintain the level of free cytokinins at a minimum. Having a minimal threshold level of cytokinins in their free form could create a situation where biochemical communication between the leaf and the root is delayed. We know that surpassing a high threshold level of cytokinins in the leaf can send a feedback signal to the roots, which inhibits cytokinin transport into the leaf. This means that a low level of free cytokinin in the leaves could induce a continuing flow of cytokinins from the roots to the leaves. This could result in the continuing active expression of key genes, for example, accumulation of rubisco, cytoplasmic glutamine synthetase, nitrite reductase, glucose-6-phosphate dehydrogenase, and chaperones, all of which would enable efficient interactions between C and N signaling pathways and would delay the onset of senescence.

C/N signaling and cytokinins in the leaves will also activate defense and receptor kinase proteins that promote disease-tolerance. Thus, the expression of genes associated with the promotion of senescence would remain underrepresented and at low levels, prolonging plant survival in its productive rather than aging phase. These genes include those for the ethylene biosynthesis enzyme-ACC synthase, cysteine protease, and the senescence-associated SAG12 gene (Kumar et al., 2004). These latter enzymes are underrepresented and remain at low levels in hairy-vetch-grown tomatoes, indicating interactions and connections between hairy-vetch residue and the growth of tomato plants.

15.6 Discussion

Crop productivity and protection are major concerns in a world that has become aware of the harmful effects to the environment and to animal and human health from excessive use of chemicals in agriculture. Reducing the use of chemicals in agriculture without having an adverse impact on the yield or quality of the crop is a reasonable objective for current agricultural research. Agriculturists will be more willing to adopt alternative practices if a strong scientific understanding has been developed of the reasons why crops cultivated under cover-crop mulches offer more net benefits. Venturing into a field situation to identify operative genetic mechanisms is daunting from a reductionist point of view because one has to deal not only with the plant itself but also with, among other things, the cover-crop residue chemistry, root physiology, and interactions of the root systems with a wide variety of microorganisms in the rhizosphere. However, unless we look at whole organisms in a field setting, as summarized here, we cannot recognize what is and what is not happening in the real world.

The employment of genetic screening, gene expression studies, and quantified protein levels using specific and sensitive methods can unambiguously demonstrate, as seen in this chapter, the existence of certain specificity inherent within the dynamic metabolic and signaling pathways offered by cover-crop-based, alternative agriculture. It is becoming apparent that many routes of communication exist between different plant organs, which are tied to receptor proteins and downstream signaling pathways. Included in these are: metabolic signals via specific metabolites when their intra- or extra-cellular levels reach some threshold, hormonal signaling via hormone receptor kinases specific to each hormone involved, and environmental signaling due to changes in the environment (Mattoo and Handa, 2004). In the real world, however, what balances these and which ones override which others still remains a matter for future discovery and definition.

Much of the work to improve N-use and C-use efficiency in crops has been carried out in isolated systems, although engineering the genes of specific proteins/enzymes, e.g., the C-fixing enzyme rubisco, glutamate-metabolizing enzymes, nitrate reductase, and photosynthetic phosphoenolpyruvate carboxylase, has generated considerable information (see Foyer and Noctor, 2002). Whatever the merits of these approaches, it is important to remain aware of the impacts that taking a more holistic view can have, focusing on common features that are defined in test tube-level experiments and at the same time reproducible in the field.

It is encouraging to see certain ideas about inter-organ signaling, e.g., root-to-leaf-to-root (Krapp et al., 2002; Sugiyama and Sakakibara, 2002) and source-to-sink for young leaves, roots, fruits (Nooden et al., 1997) — that were developed from physiological and biochemical experiments including studies on split roots, leaf discs, photosynthetic organelle, and genetic mutants — being reinforced now by our studies of tomato plants cultivated on legume cover-crop-based, alternative agriculture (Kumar et al., 2004).

Use of hairy vetch as a cover crop leads to improved vigor, increased fruit yield, delayed senescence, and suppression of weeds and pathogens when used for growing vegetables. Until recently we have known little about the mechanisms that are involved in these economically important responses. The activation of specific genes as identified above brings to the fore the importance of G × E (gene × environment) interactions in determining beneficial attributes in plants. It opens possibilities for evoking from crop plants a more productive response, not by changing genetic characteristics or by adding external inputs, but by altering plants' growing conditions.

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